

Distribution of amino acids in subdivided rat retinae

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Several authors have suggested a transmitter function for certain amino acids in the retina. These suggestions are based on studies of amino acid distribution (Kuriyama, Siskin, Haber & Roberts, 1968), release (Voaden & Starr, 1972) and uptake (Neal, Peacock & White, 1973) and on histochemical (Hyde & Robinson, 1974) and electrophysiological (Burkhardt, 1972) evidence. In the present study a method has been devised for reproducibly splitting the whole rat retina into layers allowing the distribution of amino acids in each layer to be determined. Eyes were removed from dark-adapted Wistar rats (200-300 g) of either sex under urethane anaesthesia, and the retinae rapidly dissected out in Krebs-bicarbonate at 4°C under a dim red light. Individual retinae were spread flat, frozen at -70°C and freeze-dried overnight. Each lyophilized retina was then pressed flat between the adhesive surfaces of two strips of Sellotape. Gentle separation of the strips split the retina into two layers one adherent to each piece of Sellotape. By repetition of this process with further pieces of Sellotape each retina was split into five layers each of which was then taken off its Sellotape backing in 150 µl of distilled water and homogenized. Aliquots of the homogenate were taken for protein estimation and for assay of amino acids as their ³H-dansyl derivatives as previously described (Roberts, Keen & Mitchell, 1973) modified by inclusion of ¹⁴C-labelled internal standards of eight major amino acids in each assay sample. The structure of the layers was determined by histological examination.

The concentration of every amino acid measured, except taurine, was high in the innermost layer and fell progressively toward the scleral side. This suggested that protein was an unsuitable reference for detection of a selective concentration of an amino acid in a particular

layer. The amount of each amino acid in a particular layer was therefore expressed as a percentage of the total retinal content of that amino acid. The distributions of glutamate, aspartate, serine and alanine were found to be identical. In the photoreceptor layers the proportion of taurine ($57.3 \pm 1.5\%$, mean \pm s.e., $n = 5$) was higher than that of these reference amino acids ($32.3 \pm 1.6\%$). The innermost layer, which consisted of ganglion cell, inner plexiform, inner nuclear and part of the outer plexiform layers and which could not reproducibly be further subdivided, contained proportions of glycine ($73.3 \pm 3.3\%$) and glutamine ($67.6 \pm 3.2\%$) higher than that of the reference amino acids ($53.7 \pm 1.2\%$). The proportions of γ -aminobutyric acid in this inner layer ($65.4 \pm 2.5\%$) and in the outer plexiform layer ($25.2 \pm 1.9\%$) were higher than those of the reference amino acids in these layers ($53.7 \pm 1.2\%$ and $13.9 \pm 1.0\%$ respectively). All these differences were significant at $p < 0.001$.

The selective localization of certain amino acids in specific retinal layers suggests a possible transmitter function for the amino acids in those layers.

R.A.Y. is an M.R.C. Junior Research Fellow.

References

- BURKHARDT, D.A. (1972). Effects of picrotoxin and strychnine upon electrical activity of the proximal retina. *Brain Res.*, **43**, 246-249.
- HYDE, J.C. & ROBINSON, N. (1974). Localization of sites of GABA catabolism in the rat retina. *Nature, Lond.*, **248**, 432-433.
- KURIYAMA, K., SISKIN, B., HABER, B. & ROBERTS, E. (1968). The γ -aminobutyric acid system in rabbit retina. *Brain Res.*, **9**, 165-168.
- NEAL, M.J., PEACOCK, D.G. & WHITE, R.D. (1973). Kinetic analysis of amino acid uptake by the rat retina *in vitro*. *Br. J. Pharmac.*, **47**, 656-657P.
- ROBERTS, P.J., KEEN, P. & MITCHELL, J.F. (1973). The distribution and axonal transport of free amino acids and related compounds in the dorsal sensory neuron of the rat as determined by the dansyl reaction. *J. Neurochem.*, **21**, 199-209.
- VOADEN, M.J. & STARR, M.S. (1972). The efflux of radioactive GABA from rat retina *in vitro*. *Vision Res.*, **12**, 559-566.